McrBC

1-800-632-7799
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M0272S 035140114071

M0272S

500 units	**10,000 U/ml**	**Lot: 0351401**
RECOMBINANT	**Store at –20°C**	**Exp: 7/14**

Recognition Site:

5´. . .Pu^{m}C ($N_{40\text{-}3000}$) Pu^{m}C. . . 3´

Description: McrBC is an endonuclease which cleaves DNA containing methylcytosine* on one or both strands. McrBC will not act upon unmethylated DNA (1). Sites on the DNA recognized by McrBC consist of two half-sites of the form (G/A)

New Storage Conditions

^{m}C. These half-sites can be separated by up to 3 kb, but the optimal separation is 55–103 base pairs (2,3). McrBC requires GTP for cleavage, but in the presence of a non-hydrolyzable analog of GTP, the enzyme will bind to methylated DNA specifically, without cleavage (4).

*5-methylcytosine or 5-hydroxymethylcytosine or N4-methylcytosine (5).

Source: The two component proteins are purified separately from *E. coli* K-12 strains containing plasmids encoding McrB and McrC (1).

Applications:

- CpG methylation status:
  McrBC is a tool for determining the methylation state of CpG dinucleotides (6–10). McrBC will act upon a pair of Pu^{m}CG sequence elements, thereby detecting a high proportion of methylated CpGs, but will not recognize Hpa II/Msp I sites (CCGG) in which the internal cytosine is methylated.
- Detection of cytosine-methylated DNA:
  The very short half-site consensus sequence (Pu^{m}C) allows a large proportion of the methylcytosines present to be detected. Even DNA which is not heavily methylated can be detected, as a low level of cleavage occurs even when the Pu^{m}C elements are as far as 3 kb apart.
- Enrichment for undermethylated DNA (11).

Supplied in: 300 mM NaCl, 10 mM Tris-HCl (pH 7.4), 0.1 mM EDTA, 1 mM DTT, 500 µg/ml BSA and 50% glycerol.

Reagents Supplied with Enzyme:
10X NEBuffer 2, 100X (100 mM) GTP, 100X BSA, Control Plasmid DNA.

Reaction Conditions: 1X NEBuffer 2, supplemented with 100 µg/ml BSA, 1 mM GTP. Incubate at 37°C.

1X NEBuffer 2:
50 mM NaCl
10 mM Tris-HCl
10 mM $MgCl_2$
1 mM DTT
pH 7.9 @ 25°C

Diluent Compatibility: Diluent Buffer B
300 mM NaCl, 10 mM Tris-HCl, 0.1 mM EDTA, 1 mM DTT, 500 µg/ml BSA and 50% glycerol. (pH 7.4 @ 25°C).

Unit Definition: One unit is defined as the amount of enzyme required to cleave 1 µg of a plasmid containing multiple McrBC sites in 1 hour at 37°C in a total reaction volume of 50 µl. A pilot titration of enzyme is recommended for cleavage of genomic DNA.

Quality Control Assays

16-Hour Incubation: A 50 µl reaction containing 1 µg of λ DNA (HindIII digest) and 20 units of McrBC for 16 hours at 37°C resulted in a DNA pattern free of detectable nuclease degradation as determined by agarose gel electrophoresis.

Exonuclease Activity: Incubation of a 50 ul reaction containing 25 units of McrBC with 1 µg of a mixture of single and double-stranded [^{3}H] *E. coli* DNA (10^{5} cpm/µg) for 4 hours at 37°C released < 0.1% of the total radioactivity.

Endonuclease Activity: Incubation of a 50 ul reaction containing 50 units of McrBC with 1 µg of ϕX174 RF I DNA for 4 hours at 37°C resulted in < 10% conversion to RF II as determined by agarose gel electrophoresis.

(see other side)

CERTIFICATE OF ANALYSIS

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(see other side)
CERTIFICATE OF ANALYSIS

Heat Inactivation: 100 units of enzyme were inactivated by incubation at 65°C for 20 minutes.

Notes: McrBC makes one cut between each pair of half-sites, cutting close to one half-site or the other, but cleavage positions are distributed over several base pairs approximately 30 base pairs from the methylated base (2).

Therefore the enzyme does not produce defined DNA ends upon cleavage. Also, when multiple McrBC half-sites are present in DNA (as is the case with cytosine-methylated genomic DNA) the flexible nature of the recognition sequence results in an overlap of sites, and so a smeared rather than a sharp banding pattern is produced.

McrBC cleavage of the supplied 4.3 kb linear, methylated control plasmid DNA produces several fragments between approximately 700 bp and 2.3 kb in size.

GTP is more labile than other nucleotides. We recommend aliquoting the 100 mM solution supplied and thawing and diluting as necessary.

References:

1. Sutherland, E. et al. (1992) *J. Mol. Biol.* 225, 327–334.
2. Stewart, F. J. and Raleigh E. A. (1998) *Biol. Chem.* 379, 611–616.
3. Panne, D. et al. (1999) *J. Mol. Biol.* 290, 49–60.
4. Stewart, F. J. et al. (2000) *J. Mol. Biol.* 298, 611–622.
5. Reviewed in Raleigh, E. A. (1992) *Mol. Microbiol.* 6, 1079–1086.
6. Chotai, K. A. and Payne, S. J. (1998) *J. Med. Genet.* 35, 472–475.
7. Burman, R. W. et al. (1999) *Am. J. Hum. Genet.* 65, 1375–1386.
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9. Lyko, F. et al. (2000) *Nat. Genet.* 23, 363–366.
10. Gowher, H. et al. (2000) *EMBO J.* 19, 6918–6923.
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U.S. Patent No. 5,405,760

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Page 2 (M0272)

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